Spinal Muscle Amiotrophy SMA

• Inheritance: autosomal recessive
• Gene: SMN (Survival Motor Neuron)
• Motor Neuron; Anterior Horn; Spinal Cord
• Second most common fatal autosomal recessive disorder after CF
• Second most common pediatric neuromuscular disorder after DMD
• Incidence: 1 in 6000-10000 live births
• Carrier frequency: 1 in 40-60
SMA—spinal muscular atrophy

- The spinal muscular atrophies (SMAs) are characterized by degeneration of the anterior horn cells in the spinal cord and motor nuclei in the lower brainstem.
SMN protein

Expressed in most tissues
High levels are found in spinal motor neuron
SMN exist in the cell as a part of a large complex that regulates the assembly of a specific class of RNA protein complexes - which is essential for pre-mRNA splicing. The function of SMN protein is linked to the control of protein synthesis.
PHENOTYPE CLASSIFICATION

- SMA TYPE O (congenital)
- SMA TYPE I (Werdnig-Hoffmann)
- SMA TYPE II (Werdnig-Hoffmann late)
- SMA TYPE III (Juvenile)
- SMA TYPE IV (Kugelberg-Welander)
SMA TYPE I-II

• Severe form of SMA
• Onset: first 6 months
• Death: < 2 year
• Never raising the head or sitting
SMA TYPE III

- Less severe
- Clinical appearing: < 18 months
- Able to sit unaid
- Death: about 9 years
SMA TYPE IV

- Mildest form of SMA
- Onset: > 18 months
- Walking without aid
SMA THE CLINICS

https://www.youtube.com/watch?v=dUAbrRXkWeuo
1990: The three types of SMA were mapped to 5q13

The SMA locus contains two inverted copies of a 500kb element

The two copies are named telomeric (SMN1) and centromeric (SMN2)
Three candidate genes named SMN (Survival Motor Neuron), NAIP (Neuronal Apoptosis Inhibitory Protein) and P44 were identified in this locus.
**Mutations**

- Up to **95%** of SMA patients (SMNI-III) have homozygous deletions for two exons (7&8) of both telomeric copies of the SMN gene (SMN<sup>t</sup>)

- Up to **5%** of SMA patients have frameshift mutations, gene conversions and point mutation

- Exons 5 and 6 of NAIP<sup>t</sup> gene are deleted in approximately 50% of type I SMA and 18% of types II and III SMA

- P44<sup>t</sup> is lost or interrupted in 73% of SMA type I patients and 7% in types II and III
Genetics

• All SMA patients have reduced fl-smn protein:
  – Type 1 – 9%
  – Type 2 – 14%
  – Type 3 – 18%
  – Carriers – 45 -55%

• When levels approach 23% - motor neuron function is normal.
SMN2 ESE hampers exon inclusion

A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy

Christian L. Lorson†, Eric Hahnen†‡, Elliot J. Androphy*§‡, and Brunilde Wirth‡

*Department of Dermatology, New England Medical Center, and †Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111; and ‡Institute of Human Genetics, University of Bonn, D-53111 Bonn, Germany
Genetics

Normal Individual

- SMN1
  - Ex 6
  - Ex 7
  - Ex 8
  - Presence of ESE/ Absence of ESS

- SMN2
  - Ex 6
  - Ex 7
  - Ex 8
  - Disruption of ESE/ Creation of ESS

SMN1
- 90% FL-SMN transcript
- Translation
- 90% FL-SMN protein

SMN2
- 10% FL-SMN transcript
- 90% SMN Δ7 transcript
- Translation
- 10% FL-SMN protein; SMN Δ7 protein degraded

SMA Patient

- SMN1
  - Ex 6
  - Ex 7
  - Ex 8
  - Presence of ESE/ Absence of ESS

- SMN2
  - Ex 6
  - Ex 7
  - Ex 8
  - Disruption of ESE/ Creation of ESS

SMN1
- 10% FL-SMN transcript
- 90% SMN Δ7 transcript
- Translation
- 10% FL-SMN protein; SMN Δ7 protein degraded

Insufficient FL-SMN protein for survival and maintenance of motor neurons; SMA severity dependent on SMN2 copy number

Sufficient FL-SMN protein for survival and maintenance of motor neurons
Genetics

**SMA type I**: Mutations
- Mostly SMN1 deletions
- Few missense point mutations in SMN1
- SMN2 gene copy number: Often 2

**SMA type II**
- Mutations convert SMN1 gene to SMN2
- SMN2 gene copy number: ≥ 3
- Missense point mutations more common

**SMA type III**
- SMN2 gene copy number: ≥ 3
- Missense point mutations more common

**Normal 5q chromosome**

**SMA Type 1**

**SMA Type 2**

**SMA Type 3**
SMA DIAGNOSTICS

Reason for request/clinical indication (COUNSELLING)
positive family history for SMN1 gene deletion or SMA,
partner heterozygous for the deletion,
consanguinity with the partner,
SMA clinical diagnosis.

MOLECULAR TESTS
SMN1 copy number representation among tested subjects:
74%  2 copies
14%  1 copy
  8%  3 copies
  4%  0 copies
### 2013-2016: 800 SMN1 genetic tests (MLPA) results at UNIFE

<table>
<thead>
<tr>
<th>Reason for request/clinical indication</th>
<th>n.</th>
<th>2 copies</th>
<th>1 copy</th>
<th>3 copies</th>
<th>0 copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive family history</td>
<td>480</td>
<td>347 (72.3%)</td>
<td>120 (25%)</td>
<td>13 (2.7%)</td>
<td>/</td>
</tr>
<tr>
<td>Partner heterozygous for SMN1 gene deletion</td>
<td>209</td>
<td>197 (94.2%)</td>
<td>5 (2.3%)</td>
<td>7 (3.3%)</td>
<td>/</td>
</tr>
<tr>
<td>Consanguinity with the partner</td>
<td>76</td>
<td>34 (44.7%)</td>
<td>1 (1.3%)</td>
<td>41 (53.9%)</td>
<td>/</td>
</tr>
<tr>
<td>SMA clinical diagnosis</td>
<td>35</td>
<td>3 (8.5%)</td>
<td>/</td>
<td>/</td>
<td>32 (91.5%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>800</td>
<td>73%</td>
<td>15%</td>
<td>8%</td>
<td>4%</td>
</tr>
</tbody>
</table>
MLPA, Multiplex Ligation-dependent Probe Amplification

- Denatured genomic DNA is hybridized with a mixture of 40 probes.
- Each MLPA probe consists of two oligonucleotides, one synthetic and one M13-derived.

PCR primer sequence Y

Hybridisation sequence

5' Y

3' Target A

5' Y

3' Target B

PCR primer sequence X

Stuffer sequence

Each M13 derived probe oligonucleotide has a different stuffer sequence.

The two parts of each probe hybridise to adjacent target sequences.

The two parts of hybridised probes are ligated by a thermostable ligase.

All probe ligation products are amplified by PCR using only one primer pair.

The amplification product of each probe has a unique length (130-480 bp).

Amplification products are separated by electrophoresis. Relative amounts of probe amplification products reflect the relative copy number of target sequences.
• **Diagnostic sensitivity of MLPA analysis**

• The SMA MLPA assay is a quantitative test for SMN1 gene copy number, and will not pick up subtle deletions, inversions or point mutations in SMN1 gene. Diagnostic sensitivity of the MLPA assay is additionally influenced by the fact that approximately 3-7% of the SMN1 alleles in the general population have two SMN1 copies on a single chromosome.

• Homozygous deletion of the SMN1 gene will be evident in approximately 95% of SMA Type I patients.
1 copy of SMN1 exon 7
1 copy of SMN1 exon 8
3 copies of SMN1 exons 7 and 8

0 copies of SMN1 exons 7 and 8
SMN genes sequencing

Table 1. Primer sequences and the length of the amplified SMN gene fragment

<table>
<thead>
<tr>
<th>Gene</th>
<th>SMN</th>
<th>Sequence</th>
<th>Annealing temperature, °C/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td></td>
<td>5'-3' CAGTGAATCTAGAAGATTGACGAGA</td>
<td>62/30</td>
</tr>
<tr>
<td>Exon 2A</td>
<td></td>
<td>5'-3' GTGTTGGACAGATTGAGACG</td>
<td>62/30</td>
</tr>
<tr>
<td>Exon 2B</td>
<td></td>
<td>5'-3' TGCACACCCCTGTAACATGGAC</td>
<td>62/30</td>
</tr>
<tr>
<td>Exons 3-4</td>
<td></td>
<td>5'-3' CAATCTCATCCCTCATTCCCTCAC</td>
<td>62/30</td>
</tr>
<tr>
<td>Exon 5</td>
<td></td>
<td>5'-3' GGTTTGGATCTTCTTATCCTCTATC</td>
<td>62/30</td>
</tr>
<tr>
<td>Exon 6</td>
<td></td>
<td>5'-3' GCATTCCTAGTCCATTAGAAGGACTCA</td>
<td>62/30</td>
</tr>
<tr>
<td>Exon 7</td>
<td></td>
<td>5'-3' GTGAAAGCAAAATGTTGGTAAAGATGC</td>
<td>62/30</td>
</tr>
<tr>
<td>Exon 8</td>
<td></td>
<td>5'-3' GTTGAATCGTGCAGAGGATCC</td>
<td>62/30</td>
</tr>
</tbody>
</table>

Table 2. Localization and the type of point mutations in type I–III SMA patients

<table>
<thead>
<tr>
<th>DNA no.</th>
<th>SMA type</th>
<th>Genotype</th>
<th>Mutation</th>
<th>Exon, intron</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>1T/2C</td>
<td>c.43G&gt;T</td>
<td>E1</td>
<td>de novo</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>1T/3C</td>
<td>c.684dupA</td>
<td>E5</td>
<td>From father</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>1T/2C</td>
<td>c.815A&gt;G</td>
<td>E6</td>
<td>From mother</td>
</tr>
<tr>
<td>4</td>
<td>II</td>
<td>1T/3C</td>
<td>c.821C&gt;T</td>
<td>E6</td>
<td>From mother</td>
</tr>
<tr>
<td>5</td>
<td>III</td>
<td>1T/2C</td>
<td>c.824G&gt;C</td>
<td>E6</td>
<td>From mother</td>
</tr>
<tr>
<td>6</td>
<td>III</td>
<td>1T/3C</td>
<td>c.824G&gt;C</td>
<td>E6</td>
<td>From mother</td>
</tr>
<tr>
<td>7</td>
<td>III</td>
<td>1T/2C</td>
<td>c.835-2A&gt;T</td>
<td>E7</td>
<td>From mother</td>
</tr>
<tr>
<td>8</td>
<td>III</td>
<td>1T/2C</td>
<td>c.836G&gt;T</td>
<td>E7</td>
<td>From mother</td>
</tr>
</tbody>
</table>

Zabnenkova et al. 2015

Table 3. Guidelines for the clinical indications for DNA analysis

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any reasonable suspicion of SMA</td>
<td>Homozygous SMN1 exon 7 deletion test</td>
</tr>
<tr>
<td>Clinical criteria supporting the diagnosis of SMA types I, II, or III should be in agreement with those described, including characteristic abnormalities in a muscle biopsy</td>
<td>Hemizygous SMN1 exon 7 deletion test/SMN1 subtle mutation screening</td>
</tr>
</tbody>
</table>
SMN genes sequencing

SMN exon 6

SMN exon 7
## EQA Schemes

This is a list of all the schemes your lab has registered for. **Please note:**

- If you have recently registered for a scheme and it is not in the list, then your registration is awaiting approval.
- Click on 'Details' to view the scheme details and upload your reports.
- View schemes you have registered for in other years by selecting a different year/season above, and pressing the 'Search' button.

<table>
<thead>
<tr>
<th>Details</th>
<th>Name</th>
<th>Registration Approved</th>
<th>Scheme result (SATISFACTORY or POOR)</th>
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<tbody>
<tr>
<td>Details</td>
<td>AZF</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
</tr>
<tr>
<td>Details</td>
<td>CMT</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
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<tr>
<td>Details</td>
<td>DFN1</td>
<td>10 Nov 2015 - ---</td>
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</tr>
<tr>
<td>Details</td>
<td>DMD</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
</tr>
<tr>
<td>Details</td>
<td>DNA-SEQ (Full)</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
</tr>
<tr>
<td>Details</td>
<td>FRAX (Full scheme)</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
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<tr>
<td>Details</td>
<td>FRDA</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
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<tr>
<td>Details</td>
<td>HD</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
</tr>
<tr>
<td>Details</td>
<td>PWAS</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
</tr>
<tr>
<td>Details</td>
<td>SMA</td>
<td>10 Nov 2015 - ---</td>
<td>SATISFACTORY</td>
</tr>
</tbody>
</table>

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**ISTITUTO SUPERIORE DI SANITÀ**  
Centro Nazionale Malattie Rare  
Test Genetici

**Report CEQ Beta Talassemia**  
lab4145  Performance sufficiente con punteggi medio: 13,85/14
Clinical trials and outcome measures in SMA
Therapeutic targets for SMA

- **Mutation of smn1**
- **Alternative splicing of smn2**
- **Diminution of full-length smn transcript**
- **SMN protein deficit**
- **Loss of motorneurons**
- **Clinical symptoms**

**Gene therapy**
- Replacement of SMN1
- Inclusion of exon 7
- Increase of SMN transcripts
- Stabilisation of SMN protein
- Neuroprotection
- Cell therapy

**Antisense**
- Pharmacological upregulation
  - HDAC inhibitors (Hydroxyurea, Quinazolones, Aminoglycosides)
  - Indoprofen, Proteasome inhibitors, Polyphenols
  - Riluzole, TRO19622, Neurotrophic factors
  - Stem cells

Example

*Courtesy of Francesco Muntoni*
SMA Drug Pipeline - 2014

Preclinical: Discovery
- Identification
- Optimization
- Safety & Manufacturing

Clinical Development
- Phase I
- Phase II
- Phase III

Basic Research Seed Ideas
- Trophos/Olesoxime
- ISIS/Biogen/ASO
- Pfizer/Quinazoline
- AveXis/NW/Gene Therapy
- PTC/Roche/Small Molecule
- CSC/Motor Neuron
- Paratek/Tetracycline
- NINDS/Indoprofen
- Genzyme/CNS Gene Therapy
- Novartis/Small Molecule
- CALIBR/Small Molecule
- Indiana U/Small Molecule
- OSU/UM/Morpholino ASO
- Harvard/Small Molecule
- Cytokinetics/Tirasemtiv

FDA Approval

Projects with FSMA Funding Involvement

Repurposed Drugs
- Studied for SMA
  - Valproic Acid
  - Riluzole
  - Phenytoin
  - Hydroxyurea
  - Salbutamol

ASO = Antisense Oligonucleotide
NW = Nationwide Children’s Hospital in Columbus, Ohio
CSC = California Stem Cell – On Clinical Hold
NINDS = National Institute of Neurological Disorders and Stroke
CNS = Central Nervous System

Families of SMA
- Support, Research, Hope
Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN

Kevin D Foust¹, Xueyong Wang²,³, Vicki L McGovern³,⁶, Lyndsey Braun¹, Adam K Bevan¹,⁴, Amanda M Haidet¹,⁴, Thanh T Le³, Pablo R Morales⁵, Mark M Rich², Arthur H M Burghes³,⁴ & Brian K Kaspar¹,³,⁴
Intravenous scAAV9 delivery of a codon-optimized SMN1 sequence rescues SMA mice

Elisa Dominguez1,2, Thibaut Marais1, Nicolas Chatauret1,2, Sofia Benkhelifa-Ziyyat1, Sandra Duque1, Philippe Ravassard3,4,5, Romain Carcenac1, Stéphanie Astord1, Aurélie Pereira de Moura1, Thomas Voit1 and Martine Barkats1*

A

Probability of Survival

Days

SMNdelta7

SMNdelta7 AAV9-SMN

B

Intramuscular scAAV9-SMN Injection Mediates Widespread Gene Delivery to the Spinal Cord and Decreases Disease Severity in SMA Mice

Sofia Benkhelifa-Ziyyat1, Aurore Besse1, Marianne Roda1, Sandra Duque1, Stéphanie Astord1, Romain Carcenac1, Thibaut Marais1 and Martine Barkats1

AveXis- BioLife Licenses Spinal Muscular Atrophy (SMA) Patent Portfolio from Nationwide Children’s Hospital and The Ohio State University

Columbus, OH - 10/18/2013

**Latest News**

**NIH's Recombinant Advisory Committee (RAC) Approves Infant Trial for Systemic AAV9-SMN Gene Therapy for SMA. Team Plans to Submit IND to the FDA in 2013.**

December 4, 2012.

*The Kaspar Team at Nationwide Children's Hospital presented their AAV9-SMN gene therapy program for Spinal Muscular Atrophy to the NIH RAC today. In the coming months, the team will also submit an IND to the FDA for their approval to begin human clinical trials. FSMA funding is helping move this program into older and bigger patients with SMA.*

The proposed trial would likely include infants up to six month old, who are non-ventilator dependent with a confirmed diagnosis of Type I SMA. A total of 6-10 patients will be enrolled over a period of time.
Latest News

Phase I Clinical Trial of Systemic SMN Gene Therapy For Spinal Muscular Atrophy is Open at Nationwide Children’s Hospital.

April 25, 2014.

This study is a phase I, single-site, dose escalation study to evaluate the safety and efficacy of gene transfer for Spinal Muscular Atrophy Type 1 (SMA1). The trial is enrolling infants from 0 to 9 months of age.

Click here for the trial information on www.clinicaltrials.gov.

Trial Summary

This study is a phase I, single-site, dose escalation study to evaluate the safety and efficacy of gene transfer for Spinal Muscular Atrophy Type 1 (SMA1). Enrollment is planned to begin within the first half of 2014 at Nationwide Children’s Hospital in Columbus, Ohio. Infants between 0 and 9 months of age with SMA1 may be eligible to take part in this first human trial. A total of nine patients will be enrolled to receive a one-time gene transfer infusion. Patients will continue to be monitored at Nationwide Children’s Hospital including physical exams and blood tests for two years after gene transfer.

Neurologist Jerry Mendell, MD, director, Center for Gene Therapy at Nationwide Children’s, will lead the study. A formal statement will be released soon by Nationwide Children’s Hospital.
THE Spinraza trial

• [https://www.youtube.com/watch?v=3EKs8GJnkrc](https://www.youtube.com/watch?v=3EKs8GJnkrc)

Therapeutic targets for SMA

- **Mutation of smn1**
  - Replacement of SMN1
  - Diminution of full-length smn transcript
  - SMN protein deficit
  - Loss of motorneurons
  - Clinical symptoms

- **Alternative splicing of smn2**
  - Inclusion of exon 7
  - Increase of SMN transcripts
  - Neuroprotection

- **Diminution of full-length smn transcript**
  - Stabilisation of SMN protein

- **SMN protein deficit**
  - Gene therapy

- **Loss of motorneurons**
  - Cell therapy

- **Clinical symptoms**

**Antisense**
Pharmacological upregulation

- HDAC inhibitors
  - Hydroxyurea
  - Quinazolones
  - Aminoglycosides

- Indoprofen
  - Proteasome inhibitors
  - Polyphenols

- Riluzole, TRO19622
  - Neurotrophic factors

**HDAC inhibitors**

**Pharmacological upregulation**

**Gene therapy**

**Stem cells**

*Courtesy of Francesco Muntoni*
ISIS-SMN\textsubscript{Rx}: Modulating Splicing of SMN2 to Increase Normal SMN Protein

- Uniformly 2’-O-methoxyethyl modified (MOE) antisense drug
- Corrects the splicing disorder in SMN2, resulting in the production of fully functional SMN protein in model systems
- In mild and severe mouse models of SMA provides a phenotypic and pathological benefit when delivered centrally*
- Distributes broadly to spinal cord motor neurons after intrathecal delivery in monkeys*
- Has a long half life in CNS tissue (>6 months in animal models)

Clinical Program for ISIS-SMN$_{Rx}$

- Orphan Drug Status in US and EU; Fast Track Designation in USA
- Phase 1 Single-dose Study in Children with SMA – completed
Phase 1 open label, single dose study in SMA II-III patients 2-14 years of age.

**Objective:** To evaluate the safety, tolerability, and pharmacokinetics of a single dose of ISIS-SMN$_{Rx}$ administered intrathecally to patients with Spinal Muscular Atrophy

- Single dose given intrathecally as an LP bolus injection in male and female SMA patients 2-14 years old who are medically stable
- **Primary endpoints:**
  - Safety/tolerability
  - CSF and plasma drug level pharmacokinetics
- **Exploratory efficacy endpoints included to gain experience with these endpoints**

<table>
<thead>
<tr>
<th>Cohorts</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg</td>
<td>6</td>
</tr>
<tr>
<td>3 mg</td>
<td>6</td>
</tr>
<tr>
<td>6 mg</td>
<td>6</td>
</tr>
<tr>
<td>9 mg</td>
<td>10</td>
</tr>
</tbody>
</table>

**Timeline:**
- **Open Label**
  - Day 1 Single Dose
  - Post-Treatment In-Patient f/u Period: 24 hours
  - Post-Treatment Evaluation Period:
    - 4 weeks post dose (1 mg & 3 mg)
    - 12 weeks post dose (6 mg & 9 mg)
- **Screening** (≤28 days)
Safety and Tolerability Results

- **ISIS-SMN$_{Rx}$** was well tolerated, with no significant safety findings when given as a single dose up to 9 mg
  - No SAEs or potential Dose Limiting Toxicities
  - Adverse Events all mild (67/72 AEs) or moderate (5/72 AEs) in severity
  - Adverse Events were not related to dose level (see next slide)
  - No drug-related changes on neurological exams
  - No changes in CSF safety labs or CSF cytokines (IL6, TNF-alpha, MCP1) compared to pre-dose (at 7 days post-dose for Cohorts 1-3; for Cohort 4 at 7 or 28 days post-dose)

- The LP injection procedure in SMA children was also well tolerated and was shown to be feasible
CSF and plasma drug levels were reasonably consistent with predicted values from monkey nonclinical studies.
Increases in HFMSE Scores Observed in the Phase 1 Study up to 14 Months After a Single Dose

1-3 Months Post Single Dose
- At Day 85, mean change from baseline = 3.1 points (p=0.02)
- 6/10 patients with change ≥ 4 points

9-14 Months Post Single Dose
- At 9-14 months, mean change from baseline = 5.75 points (p=0.008)
- No patients declined
- 6/8 patients with change ≥ 4 points

Observations in the 9 mg cohort
- At Day 85, mean change from baseline = 3.1 points (p=0.02)
- 6/10 patients with change ≥ 4 points
Clinical Program for ISIS-SMN$_{\text{Rx}}$

- Orphan Drug Status in US and EU; Fast Track Designation in USA
- **Phase 1 Single-dose** Study in Children with SMA – completed

- **Phase 1b/2a Multiple-dose** Study in Children with SMA - ongoing

- **Phase 2 Multiple-dose** Study in Infants with SMA - ongoing

- Two pivotal, controlled studies planned to start in 2014, to be conducted worldwide, including Europe
  - Phase 2/3 study in Infants with SMA
  - Phase 2/3 study in Children with SMA
Pharmacological modification of SMN2 splicing

In vitro activity of SMN2 splicing modifiers

SMN protein increase in Type I SMA fibroblasts
Therapeutic effects of SMN2 splicing modifiers

Profound efficacy in Δ7 mice, a model for Type I SMA
Therapeutic targets for SMA

- **Mutation of smn1**
- **Alternative splicing of smn2**
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- **Stabilisation of SMN protein**
- **Neuroprotection**
- **Cell therapy**

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- **Antisense**
  - Pharmacological upregulation
  - HDAC inhibitors
  - Hydroxyurea
  - Quinazolones
  - Aminoglycosides

**Drug therapy**
- Indoprofen
- Proteasome inhibitors
- Polyphenols
- Riluzole, **TRO19622**

**Stem cells**

**Stem cells**

**Courtesy of Francesco Muntoni**
Discovery/Optimization of Quinazolines for Potential Treatment of SMA

SMN2 promoter assay-based HTS


Hit/Lead optimization by DeCODE Chemistry Inc. using promoter assay


DcpS (scavenger decapping enzyme) identified as putative target of C5-substituted quinazolines by deCODE Chemistry Inc.


Demonstration of increased Smn promoter activity in vivo in CNS of SMA mice by 2,4-diaminoquinazoline derivatives and increased in survival by D-156844


Increase of SMN transcripts

![D156844](image)
Discovery/Optimization of Quinazolines for Potential Treatment of SMA

- 2009: Program licensed from Families of SMA to RepliGen
- RepliGen selects D157495 (RG3039) as clinical lead
- 2011: RG3039 enters the clinic supported by a grant from Muscular Dystrophy Association
- Beneficial effects of RG3039 demonstrated in 3 different SMA mouse models
  - Gogliotti et al. (2013) *HMG.* 22(20):4084-101
  - Van Meerbeke et al. (2013) *HMG.* 22 (20) : 4074-83
- Jan 2013: Announcement of program being licensed from RepliGen to Pfizer
Therapeutic targets for SMA

Mutation of \textit{smn1} \quad \leftrightarrow \quad \text{Replacement of SMN1} \quad \rightarrow

Alternative splicing of \textit{smn2} \quad \leftrightarrow \quad \text{Inclusion of exon 7} \quad \rightarrow

Diminution of full-length \textit{smn} transcript \quad \leftrightarrow \quad \text{Increase of SMN transcripts} \quad \rightarrow

\text{SMN protein deficit} \quad \leftrightarrow \quad \text{Stabilisation of SMN protein} \quad \rightarrow

Loss of motorneurons \quad \leftrightarrow \quad \text{Neuroprotection} \quad \rightarrow

\text{Clinical symptoms} \quad \leftrightarrow \quad \text{Cell therapy} \quad \rightarrow

\textit{Gene therapy}

\text{Antisense}
\text{Pharmacological upregulation}

- HDAC inhibitors
- Hydroxyurea
- Quinazolones
- Aminoglycosides

- Indoprofen
- Proteasome inhibitors
- Polyphenols

- Riluzole, \text{TRO19622}
- Neurotrophic factors

\text{Stem cells}

\textit{Courtesy of Francesco Muntoni}
A clinical trial of olesoxime (TRO19622) in SMA

Study sites: 22 sites in France, Germany, Italy, UK, Poland, Netherlands, Belgium

Total number of subjects: 150 patients in total, 100 in the olesoxime group, and 50 in the placebo group.

Study duration: Total of 33 months with a 9 months recruitment period and a 2 years treatment period

Outcome of the study expected 4Q2013
Olesoxime 10 mg/kg (N=100)

Futility analysis positive

Randomisation
2 olesoxime/1 placebo

Placebo (N=50)

Screening
HMFS

1-4 Weeks

Day 0
MFM
CMAP

V1
PK
DMC

T1

V2
PK
HFMS
DMC

V3
PK
MFM
CMAP
DMC

V4
HFMS
DMC

V5
PK
MFM
CMAP
DMC

V6
HFMS
DMC

V7
PK
MFM
CMAP
DMC

V8
HFMS
DMC

V9
PK
MFM
CMAP
DMC

104-weeks Treatment Period

Interim efficacy positive: all patients get active treatment

STOP
**DEMOGRAPHIC DATA**

<table>
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<th>TRO</th>
<th>PBO</th>
<th>Overall</th>
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<tbody>
<tr>
<td><strong>Gender: Male:</strong></td>
<td>53.4%</td>
<td>43.9%</td>
<td>50%</td>
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<tr>
<td><strong>Age class: Less than 6 years old:</strong></td>
<td>34.0%</td>
<td>24.6%</td>
<td>30.6%</td>
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<tr>
<td><strong>Age: median</strong></td>
<td>7 yrs</td>
<td>11 yrs</td>
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<td><strong>SMA Type:</strong></td>
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- The two treatment groups are not perfectly comparable on the gender and age characteristics.
- The effect of gender and age, as well as SMA Type or Country on the outcome was analysed. None of these factors influenced the observed treatment effect at Month 24 (Primary Endpoint).
SECONDARY ENDPOINT – MFM TOTAL SCORE

- Magnitude of the treatment effect at M24: 2.21% [0.04; 4.38]
- The treatment effect at M24 is significantly in favour of olesoxime (p=0.0463)
- **Conclusion:** no change in the interpretation if we consider the MFM total score instead of MFM D1+D2
Olesoxime delays motor function loss for at least 2 years when analysing the change over time on the MFM motor scale.

Analysis of HFMS confirms these results, even though if it does not reach statistical significance at month 21.

Both motor scales show similar results when focusing on the patients that do not show worsening over the study (responder analysis). Significance level is reached when using the HFMS.

The good safety profile of the drug is confirmed.

Fewer AEs relative to the underlying disease have been reported in the olesoxime arm, compared to placebo group (e.g., respiratory infections and orthopedic procedures).

Overall, the results are very encouraging and will be presented to the regulatory authorities as soon as the dossier is complete.

E. Mercuri a,*, A. Mayhew b, F. Muntoni b, S. Messina a, V. Straub c, G.J. Van Ommen d, T. Voit e, E. Bertini f, K. Bushby c, On behalf of the TREAT-NMD Neuromuscular Network

ICC survey on outcome measures (2008)
ICC Survey Parameters
Sent to International SMA Community (ICC and TREAT-NMD lists) in February, 2008
35 Sites responded
22 Countries were represented
Fig. 2. Correlation of the HFMS and MFM20 baseline scores.

Hammersmith Functional Motor Scale and Motor Function Measure-20 in non ambulant SMA patients

E. Mazzone\textsuperscript{a}, R. De Sanctis\textsuperscript{a}, L. Fanelli\textsuperscript{a}, F. Bianco\textsuperscript{a}, M. Main\textsuperscript{b}, M. van den Hauwe\textsuperscript{c}, M. Ash\textsuperscript{b}, R. de Vries\textsuperscript{d}, J. Fagoaga Mata\textsuperscript{e}, K. Schaefer\textsuperscript{f}, A. D’Amico\textsuperscript{g}, G. Colia\textsuperscript{g}, C. Palermo\textsuperscript{a}, M. Scoto\textsuperscript{b}, A. Mayhew\textsuperscript{h}, M. Eagle\textsuperscript{a}, L. Servais\textsuperscript{i}, M. Vigo\textsuperscript{a}, A. Febrer\textsuperscript{e}, R. Korinthenberg\textsuperscript{f}, M. Jeukens\textsuperscript{d}, M. de Viess\textsuperscript{d}, A. Totoescu\textsuperscript{b}, T. Voit\textsuperscript{i}, K. Bushby\textsuperscript{h}, F. Muntoni\textsuperscript{b}, N. Goemans\textsuperscript{c}, E. Bertini\textsuperscript{g}, M. Pane\textsuperscript{a}, E. Mercuri\textsuperscript{a,n}
Goemans et al, 2013

Observed Natural History Data

Italian network Mazzone 2012, 2013

Ataluren trial N=57
McDonald et al. 2013

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Goemans et al, 2013

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